Emergence of Domestically Acquired Ceftriaxone-Resistant *Salmonella* Infections Associated With AmpC β-Lactamase

Eileen F. Dunne, MD, MPH
Paul D. Fey, PhD
Pat Kludt, MPH
Roshan Reporter, MD, MPH
Farzad Mostashari, MD, MSPH
Pam Shillam, MPH
Julie Wicklund, MPH
Corinne Miller, MPH
Ben Holland, BS
Karen Stamey, BS
Timothy J. Barrett, PhD
James K. Rasheed, PhD
Fred C. Tenover, PhD
Efrain M. Ribot, PhD
Frederick J. Angulo, DVM, PhD

Context Ceftriaxone, an expanded-spectrum cephalosporin, is an antimicrobial agent commonly used to treat severe *Salmonella* infections, especially in children. Ceftriaxone-resistant *Salmonella* infections have recently been reported in the United States, but the extent of the problem is unknown.

Objectives To summarize national surveillance data for ceftriaxone-resistant *Salmonella* infections in the United States and to describe mechanisms of resistance.

Design and Setting Case series and laboratory evaluation of human isolates submitted to the Centers for Disease Control and Prevention from 17 state and community health departments participating in the National Antimicrobial Resistance Monitoring System (NARMS) for enteric bacteria between 1996 and 1998.

Patients Patients with ceftriaxone-resistant *Salmonella* infections between 1996 and 1998 were interviewed and isolates with decreased ceftriaxone susceptibility were further characterized.

Main Outcome Measures Exposures and illness outcomes, mechanisms of resistance.

Results The prevalence of ceftriaxone-resistant *Salmonella* was 0.1% (1 of 1326) in 1996, 0.4% (5 of 1301) in 1997, and 0.5% (7 of 1466) in 1998. Ten (77%) of the 13 patients with ceftriaxone-resistant infections were aged 18 years or younger. The patients lived in 8 states (California, Colorado, Kansas, Massachusetts, Maryland, Minnesota, New York, and Oregon). Nine (82%) of 11 patients interviewed did not take antimicrobial agents and 10 (91%) did not travel outside the United States before illness onset. Twelve of the 15 *Salmonella* isolates with ceftriaxone minimum inhibitory concentrations of 16 µg/mL or higher were serotype Typhimurium but these isolates had different pulsed-field gel electrophoresis patterns. Thirteen of these 15 isolates collected between 1996 and 1998 were positive for a 631–base pair polymerase chain reaction product obtained by using primers specific for the *ampC* gene of *Citrobacter freundii*.

Conclusions Domestically acquired ceftriaxone-resistant *Salmonella* has emerged in the United States. Most ceftriaxone-resistant *Salmonella* isolates had similar AmpC plasmid-mediated resistance.

JAMA. 2000;284:3151-3156 www.jama.com

EACH YEAR, AN ESTIMATED 1.4 MILLION persons in the United States are infected with *Salmonella*.[1] Many of these infections occur in children; 7% of the 2840 culture-confirmed *Salmonella* infections reported to the Centers for Disease Control and Prevention in 1998 through Foodborne Diseases Active Surveillance Network (FoodNet) occurred in persons aged 18 years or younger.[2] Although most *Salmonella* infections result in mild-to-moderate gastroenteritis, which resolves spontaneously, systemic infections, including bacteremia and meningitis, also occur.[3] Invasive infections commonly occur in children, particularly in infants.[4] Ten percent of culture-confirmed infections from blood or the central nervous system reported to the Centers for Disease Control and Prevention were in infants younger than 1 year. Furthermore, an estimated 595 persons die each year as a consequence of *Salmonella* infections.[1]

Antimicrobial agents are commonly used empirically for the treatment of patients with moderate-to-severe diar-

Author Affiliations are listed at the end of this article.
Corresponding Author and Reprints: Frederick J. Angulo, DVM, PhD, Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS A-38, Atlanta, GA 30333 (e-mail: FAngulo@cdc.gov).

©2000 American Medical Association. All rights reserved.

rhea and may be lifesaving for persons with systemic *Salmonella* infections. Fluoroquinolones (eg, ciprofloxacin) are often used for treating adults with *Salmonella* infections but are not approved for use in children in the United States. Children with *Salmonella* infections, particularly invasive infections, are commonly treated with expanded-spectrum cephalosporins (eg, ceftriaxone) because of their favorable pharmacokinetic properties and low prevalence of resistance.[5-8] A limited number of cases of ceftriaxone-resistant *Salmonella* have been reported from South America, North Africa, Asia, Europe (France, Spain, Turkey, Greece, Hungary, United Kingdom, and Russia), and the United States.[9-16] A national survey of 10% of culture-confirmed *Salmonella* infections in 1995 found 3 of 4003 isolates resistant to ceftriaxone; each of these infections was acquired outside the United States.[17] A recent article reported the first case of a domestically acquired ceftriaxone-resistant *Salmonella* infection in the United States in a child who apparently acquired the infection from cattle.[16] We reviewed national surveillance data to determine the magnitude and molecular mechanisms of this emerging problem.

METHODS

Surveillance and Antimicrobial Susceptibility Testing

After serotyping, public health laboratories in the 17 participating state and community health departments in the National Antimicrobial Resistance Monitoring System, the national surveillance system for antimicrobial resistance in enteric bacteria, forwarded every 10th *Salmonella* isolate received at their laboratory between 1996 and 1998 to the Centers for Disease Control and Prevention for antimicrobial-susceptibility testing. The population served by the 17 sites in 1998 was 97 million persons, 37% of the US population. Minimum inhibitory concentrations (MICs) were determined for amikacin, ampicillin, amoxicillin-clavulanic acid, apramycin, ceftiofur (an extended-spectrum cephalosporin used in veterinary medicine), ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, and trimethoprim-sulfamethoxazole by using the Sensititre broth microdilution system (Trek Diagnostics, Westlake, Ohio). When established, National Committee for Clinical Laboratory Standards were used; resistance to ceftiofur and apramycin was defined as MICs of 8 µg/mL or higher and 32 µg/mL or higher, respectively. Isolates collected between 1996 and 1998 with decreased susceptibility to ceftriaxone (MIC ≥16 µg/mL) were confirmed as *Salmonella* and tested for resistance to cefoxitin, cefotaxime, and ceftazidime by Episolometer test (E-test) according to the manufacturer's recommendations (AB-Biodisk, Solona, Sweden), for the full range of MICs for ceftriaxone by broth microdilution according to the National Committee for Clinical Laboratory Standards, and by further molecular characterization.[18] Ceftriaxone-resistant *Salmonella* isolates had MICs of 64 µg/mL or higher and intermediate isolates had MICs of 16 to 32 µg/mL by broth microdilution. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

With the assistance of state and local health departments, we attempted to interview patients with ceftriaxone-resistant *Salmonella* infections. Using a standard questionnaire, we asked patients about their illness, including symptoms, clinical outcomes, and hospitalization. Patients were also asked about activities before illness onset, including use of antimicrobial agents for another illness, farm visitation, and international travel in the 5 days before illness onset.

Molecular Characterization

Salmonella serotype Typhimurium isolates collected between 1996 and 1998 were subtyped by bacteriophage typing[19] and pulsed-field gel electrophoresis.[20] The molecular mechanism of ceftriaxone resistance among *Salmonella* isolates with MICs of 16 µg/mL or higher were characterized by β-lactamase extraction and isoelectric focusing, amplification of the *ampC* gene from *Citrobacter freundii* by polymerase chain reaction, plasmid profile analysis, and conjugal mating experiments with *E coli* C600N. Crude preparations of β-lactamases produced by these isolates were obtained by submitting cells grown in 100 mL of Trypticase soy broth (Difco, Detroit, Mich) to 5 cycles of freezing in 0.2-mol sodium acetate (Sigma, St Louis, Mo) and thawing followed by centrifugation.

Isoelectric focusing was performed at room temperature on a Bio-Rad mini isoelectric focusing 111 (Bio-Rad, Richmond, Calif). Enzymes were visualized by activity staining after the gel was overlaid with a 500-µg/mL solution of nitrocephin (BBL, Cockeysville, Md).[21] The isoelectric points of enzymes from the *Salmonella* isolates were estimated by comparison with TEM-1, TEM-10, SHV-5, and P99 β-lactamases.

Plasmid DNA was isolated by previously described methods.[22] The polymerase chain reaction primers used to determine the presence of the *ampC* gene from *Citrobacter freundii* have been previously described.[10,11] The double-disk diffusion assay, using ceftriaxone, ceftazidime, cefotaxime, cefoxitin, and amoxicillin-clavulanic acid as indicator antimicrobial agents, was used to screen for the presence of extended-spectrum β-lactamases according to previously published methods.[23] All *Salmonella* isolates (donor strain; ampicillin-resistant, nalidixic acid-susceptible), excluding 1 isolate that was nalidixic acid–resistant, were mated to *E coli* C600N[24] (recipient strain, ampicillin-susceptible, nalidixic acid–resistant) using standard methods.[11] Counter selection was performed on Luria-Bertoni Broth agar containing 50 µg/mL of ampicillin and 30 µg/mL of nalidixic acid (Difco).

RESULTS

Fifteen (0.4%) of 4093 *Salmonella* isolates submitted from 1996 to 1998 had ceftriaxone MICs of 16 µg/mL or higher; 13 were resistant (MIC ≥64 µg/mL) and

©2000 American Medical Association. All rights reserved.

2 were intermediate (MIC, 16-32 µg/mL). The prevalence of ceftriaxone-resistant *Salmonella* was 0.1% (1 of 1326) in 1996, 0.4% (5 of 1301) in 1997, and 0.5% (7 of 1466) in 1998. Twelve (80%) of the 15 *Salmonella* isolates with ceftriaxone MICs of 16 µg/mL or higher were *Salmonella* serotype Typhimurium, the other isolates were serotypes Newport, Thompson, and Cubana. Seven of the 12 *Salmonella* Typhimurium isolates did not react with any of the typing phages (untypable), 2 isolates reacted with phages but did not conform to any defined pattern (reacts but does not conform), 2 isolates were definitive type 104 (DT104), and 1 was definitive type 21 (TABLE).

None of these *Salmonella* Typhimurium isolates had a pulsed-field gel electrophoresis pattern that was indistinguishable from that of another isolate in the group. Even the 2 DT104 isolates (FIGURE, lanes 10 and 14) had highly similar but not identical pulsed-field gel electrophoresis patterns.

Each of the 13 ceftriaxone-resistant isolates was also resistant to ampicillin, cephalothin, and ceftiofur; 12 (92%) were resistant to amoxicillin-clavulanic acid. Some ceftriaxone-resistant isolates were additionally resistant to aminoglycosides (92% to streptomycin, 62% to kanamycin, and 23% to gentamicin), sulfonamides (77%), tetracycline (77%), and chloramphenicol (69%). All ceftriaxone-resistant isolates were susceptible to amikacin, apramycin, and ciprofloxacin. One isolate was resistant to nalidixic acid and had decreased susceptibility to ciprofloxacin (MIC, 0.12 µg/mL). All 13 ceftriaxone-resistant isolates collected between 1996 and 1998 were intermediate or resistant to ceftazidime and cefotaxime.

Epidemiologic Results

All 13 ceftriaxone-resistant isolates were from stool cultures. Patients lived in 8 states (California, Colorado, Kansas, Massachusetts, Maryland, Minnesota, New York, Oregon). The age range of the patients was younger than 1 year to 52 years (age missing for 1 patient); 10 (77%) were 18 years or younger and 5 (38%) were aged 1 year or younger.

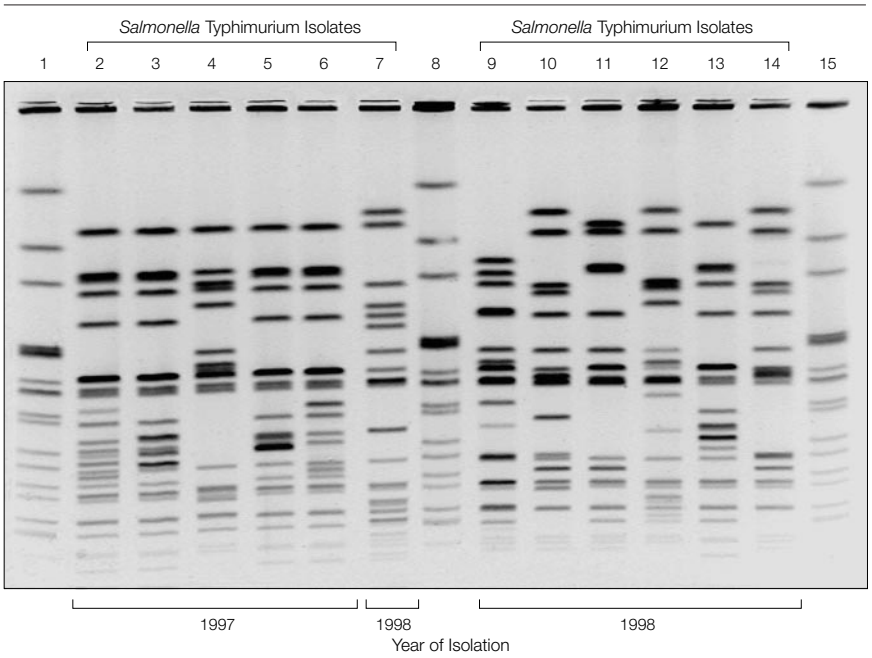
Ten of 11 interviewed patients with a ceftriaxone-resistant *Salmonella* infection reported having gastrointestinal tract symptoms. Patients with gastrointestinal tract symptoms reported having diarrhea (82%), abdominal cramps (82%), fever (73%), blood in stool (55%), and vomiting (36%). Du-

Table. Characteristics of *Salmonella* Isolates*

Isolate No.	Year of Isolation	Ceftriaxone MICs, µg/mL	NCCLS Interpretation†	Patient Age, y	Serotype	Phage Type‡
AM01358	1996	64	Resistant	NA	Thompson	
AM02039	1997	>64	Resistant	18	Typhimurium	RDNC
AM02152	1997	>64	Resistant	1	Typhimurium	Untypable
AM02544	1997	>64	Resistant	<1	Typhimurium	Untypable
AM02668	1997	>64	Resistant	6	Typhimurium	Untypable
AM02855	1997	>64	Resistant	8	Typhimurium	Untypable
AM03430	1998	>64	Resistant	<1	Typhimurium	Untypable
AM03977	1998	>64	Resistant	1	Typhimurium	Untypable
AM04204	1998	>64	Resistant	<1	Typhimurium	DT104
AM04287	1998	32	Intermediate	19	Typhimurium	DT21
AM04501	1998	>64	Resistant	38	Typhimurium	Untypable
AM04528	1998	64	Resistant	15	Newport	
AM04656	1998	64	Resistant	52	Typhimurium	DT104
AM04255	1998	32	Intermediate	32	Typhimurium	RDNC
AM04707	1998	>64	Resistant	4	Cubana	

*MICs indicates minimum inhibitory concentrations; NCCLS, National Committee for Clinical Laboratory Standards; and NA, not available. The only isolate whose source of specimen was not stool was AM04255, which came from a wound source.
†Isolate indicated as intermediate have MICs of 16 to 32 µg/mL; resistant, 64 µg/mL or higher.
‡Phage type only available for serotype Typhimurium. RDNC indicates reacts but does not conform.

Figure. Pulsed-Field Gel Electrophoresis Patterns of *Salmonella* Typhimurium Isolates With Ceftriaxone Minimum Inhibitory Concentrations of 16 µg/mL or Higher



Lanes 1, 8, and 15: molecular size standard; lane 2: AM02039; lane 3: AM02152; lane 4: AM02544; lane 5: AM02668; lane 6: AM02855; lane 7: AM03430; lane 9: AM03977; lane 10: AM04204; lane 11: AM04255; lane 12: AM04287; lane 13: AM04501; and lane 14: AM04656.

©2000 American Medical Association. All rights reserved.

ration of diarrhea ranged from 1 to 14 days, with a median of 5 days. All interviewed patients sought medical care. Six of these patients (55%) reported that the *Salmonella* infection caused interference with daily activities. The number of days of illness ranged from 1 to 14 days (median, 4 days). Five patients (45%; age range, <1-52 years) received antimicrobial agents for their *Salmonella* infection (3 received ciprofloxacin, none received cephalosporins), and 3 patients (27%) received intravenous fluids. None of the patients took an antimicrobial agent after illness onset but before collection of the specimen that yielded ceftriaxone-resistant *Salmonella*. Three patients (27%) were hospitalized; the length of hospitalization ranged from 1 to 5 days. No patients had an invasive infection. One patient was evaluated with colonoscopy. None of the patients died.

Ten (91%) of the 11 patients interviewed with ceftriaxone-resistant *Salmonella* infection reported being in good health before their *Salmonella* infection; 1 patient reported an underlying immunocompromising condition. Nine (82%) of the 11 patients interviewed did not take antimicrobial agents for another illness in the 5 days before *Salmonella* illness onset. Of the 2 interviewed patients who took an antimicrobial agent, 1 took a cephalosporin (cephalexin). Ten (91%) of the 11 patients did not travel outside the United States before illness onset. Two patients (18%) visited a farm during the 5 days before illness onset; farm animals included horses, cows, chickens, and pigs. One patient drank unpasteurized milk in the 5 days before his illness onset.

Characterization of Resistance Genes

Thirteen of 15 *Salmonella* isolates with ceftriaxone MICs of 16 µg/mL or higher from 1996 to 1998 were cefoxitin-resistant and were phenotypically classified as having an AmpC β-lactamase based on a negative double-disk diffusion test result. The double-disk diffusion test detects whether a β-lactamase is affected by the β-lactamase inhibitor clavulanic acid. These 13 isolates, which included 2 intermediate isolates (MIC, 32 µg/mL), also expressed a β-lactamase with an isoelectric point of more than 9.0, further suggesting that the ceftriaxone-resistance was mediated by an AmpC β-lactamase. Primers specific for the *ampC* gene of *Citrobacter freundii* amplified an appropriate 631–base pair product in all 13 of these isolates. These same primers were used to amplify and characterize a CMY-2 β-lactamase recently found in a ceftriaxone-resistant *Salmonella* Typhimurium from Nebraska.[16] Six of these 13 isolates also expressed a TEM-1-like β-lactamase with an isoelectric point of 5.4. Confirming this observation, all 6 isolates were positive for an expected 952–base pair polymerase chain reaction product using primers specific for the TEM family of β-lactamases. Five of the 13 *Salmonella* isolates were able to transfer ceftriaxone resistance to *E coli* C600N. All C600N transconjugants expressed the AmpC β-lactamase (isoelectric point >9.0), which suggested that this β-lactamase was mediating the ceftriaxone resistance and was encoded on a transferable plasmid. The transferable plasmids ranged from 160 to 170 kilobase. The remaining 2 ceftriaxone-resistant *Salmonella* isolates (AMO2544 and AMO4707) expressed a putative extended-spectrum β-lactamase as assessed by a positive double-disk diffusion test result. Isolate AMO2544 expressed 2 β-lactamases, 1 with an isoelectric point of 5.4 and the other with an isoelectric point of approximately 8.0. Isolate AMO4707 also expressed 2 β-lactamases with isoelectric points of 5.4 and approximately 7.6.

COMMENT

Ceftriaxone resistance has emerged among *Salmonella* isolates in the United States. In 1998, 0.5% of *Salmonella* isolates tested were resistant to ceftriaxone. Since there are an estimated 1.4 million *Salmonella* infections annually in the United States, this suggests that several thousand *Salmonella* infections each year are caused by a ceftriaxone-resistant strain. Although antimicrobial agents are not indicated for the treatment of uncomplicated salmonellosis, ceftriaxone is commonly used empirically to treat children with severe *Salmonella* infections, including *Salmonella* bacteremia and meningitis. Continued emergence and dissemination of ceftriaxone resistance, therefore, is likely to have clinical consequences.[25,26]

Ceftriaxone-resistant *Salmonella* infections are now being acquired domestically in the United States. This is in contrast to a large national survey in 1995, which reported no domestically acquired ceftriaxone-resistant infections.[17] Furthermore, no cephalosporin resistance was present in the previous national study conducted in 1990.[27] In our study, 10 (91%) of 11 interviewed persons with ceftriaxone-resistant infections reported no recent international travel.

The sources of the ceftriaxone-resistant infections described here are not known, but as with other *Salmonella* infections they are likely to be contaminated food, particularly foods of animal origin. Ceftriaxone resistance is evident in *Salmonella* isolates, particularly Typhimurium, from food-producing animals in the United States; in 1998, 2 (2%) of 98 *Salmonella* Typhimurium isolates collected from chickens and cattle in slaughter plants were resistant to ceftriaxone.[28] Recent reports of ceftriaxone-resistant *Salmonella* from cattle in Nebraska and a subsequent human infection and ceftriaxone-resistant *Salmonella* in pigs, cattle, and humans in Iowa are additional evidence of a food-producing animal reservoir of ceftriaxone-resistant *Salmonella*.[16,29] Interestingly, in our investigation, 2 (18%) of 11 interviewed patients visited a farm in the 5 days before their illness onset and 1 of those patients consumed unpasteurized milk. Furthermore, 2 ceftriaxone-resistant *Salmonella* isolates were DT104, a strain of Typhimurium that has caused illness in food-producing animals and humans in the United States.[30]

The emergence of ceftriaxone resistance is occurring among several strains of *Salmonella* rather than through the

©2000 American Medical Association. All rights reserved.

emergence of a single clone. This suggests horizontal dissemination of a resistance determinant. Our demonstration of conjugal transfer of the ceftriaxone-resistance determinant by some of the ceftriaxone-resistant isolates also supports this hypothesis.

In contrast to ceftriaxone-resistant isolates outside the United States, which have involved TEM or SHV-derived extended-spectrum β-lactamases, most of the ceftriaxone-resistant determinants in our study were due to an AmpC-type resistance gene in the BIL-1, LAT1, or CMY2 family of cephamycinases.[31,32] This finding of a similar molecular mechanism of resistance among different strains of *Salmonella* supports horizontal dissemination. Interestingly, the recent reports of ceftriaxone-resistant *Salmonella* in Nebraska[16] and Iowa[29] also had an AmpC mechanism of resistance (CMY-2). AmpC plasmid-mediated β-lactamases have been reported in clinical strains of *Klebsiella pneumoniae*, *E coli*, and *Enterobacter aerogenes*, but rarely in *Salmonella* from the United States.[9,10-14,31-33]

AmpC β-lactamases have been recently described among *E coli* isolates from calves with diarrhea in the United States[34]; although treatment histories were not described, the authors suggest that the emergence of AmpC-mediated resistance may have been a consequence of use of ceftiofur in the calves. Our demonstration of cross-resistance between ceftiofur and ceftriaxone supports a similar conclusion. Ceftiofur is the only cephalosporin approved for systemic use in food-producing animals in the United States. This antimicrobial was approved for therapeutic use in cattle by injection in the United States in 1988, and is now also approved for similar use in pigs, sheep, chicken, and turkeys. Ceftiofur use other than labelled indications is also permitted; for example, ceftiofur is frequently injected into chicken eggs prior to hatching on a flock-wide basis for disease prevention.

Ceftriaxone is a standard therapy for serious *Salmonella* infections in children. Although most persons with *Salmonella* infection do not require antimicrobial therapy, it can be lifesaving for persons with invasive disease. Unfortunately, resistance to ceftriaxone probably will continue to increase in *Salmonella*. To mitigate potential rapid dissemination, there is a need to control selective pressure in human and particularly veterinary medicine by reducing the misuse and overuse of extended-spectrum cephalosporins. Efforts to reduce the transmission of *Salmonella* to humans through the food supply, such as the new regulations for safe processing of meat and poultry to decrease the prevalence of *Salmonella* in these products and enhanced food safety education programs, also need to be emphasized.

Studies are needed to further determine the association between use of ceftiofur in food-producing animals and the emergence and dissemination of ceftriaxone resistance, particularly resistance caused by an *ampC* resistance gene. Because there is cross-resistance between ceftiofur and ceftriaxone, and food-producing animals are the source of most domestically acquired *Salmonella* infections, the use of ceftiofur in food-producing animals apparently is contributing to ceftriaxone-resistant *Salmonella*, which is transmitted to consumers through the food supply.

To protect the public's health, acceptable thresholds for ceftriaxone resistance in *Salmonella* should be established that are consistent with the framework proposed in December 1998 by the US Food and Drug Administration for evaluating the human safety of antimicrobial agents in food-producing animals.[35] Limitations on expanded-spectrum cephalosporin use in food-producing animals may be indicated, particularly if such resistance increases.

ADDENDUM

Added at Press Time

Recently available data indicate that in 1999, 28 (1.9%) of 1499 *Salmonella* isolates submitted to the National Antimicrobial Resistance Monitoring System had ceftriaxone MIC of 16 µm/mL or higher, indicating decreased susceptibility to ceftriaxone. Full-range MIC testing by broth microdilution, epidemiologic investigation, and molecular characterization is pending.

Author Affiliations: Foodborne and Diarrheal Diseases Branch, Division of Bacterial and Mycotic Diseases (Drs Dunne, Barrett, Rasheed, Ribot, and Angulo, and Mr Holland and Ms Stamey) and Hospital Infections Program, Nosocomial Pathogens Laboratory Branch (Dr Tenover), National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Ga; Section of Infectious Diseases, University of Nebraska Medical Center, Omaha (Dr Fey); Massachusetts Department of Public Health, Boston (Ms Kludt); Los Angeles County Health Department, Los Angeles, Calif (Dr Reporter); New York City Department of Health, New York, NY (Dr Mostashari); Colorado Department of Public Health and Environment, Denver (Ms Shillam); Minnesota Department of Health, St Paul (Ms Wicklund); and the Kansas Department of Health and Environment, Topeka (Ms Miller).

Funding/Support: Funding for this project was through the National Antimicrobial Resistance Monitoring System for enteric bacteria.

Acknowledgment: We thank Shannon Rossiter, MPH, for her help with data analysis.

REFERENCES

1. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis.* 1999;5:607-625.

2. Centers for Disease Control and Prevention, FoodNet 1998 Web site. Available at: http://www.cdc.gov/ncidod/dbmd/foodnet. Accessed November 20, 2000.

3. Torrey S, Fleisher G, Jaffe D. Incidence of *Salmonella* bacteremia in infants with *Salmonella* gastroenteritis. *J Pediatr.* 1986;108:718-721.

4. Sirinavin S, Jayanetra P, Thakkinstian A. Clinical and prognostic categorization of extraintestinal nontyphoidal *Salmonella* infections in infants and children. *Clin Infect Dis.* 1999;29:1151-1156.

5. Stutman HR. *Salmonella*, *Shigella*, and *Campylobacter*: common bacterial causes of infectious diarrhea. *Pediatr Ann.* 1994;23:538-543.

6. Cherubin CE, Eng RH, Smith SM, Goldstein EJ. Cephalosporin therapy for salmonellosis. *Arch Intern Med.* 1986;146:2149-2152.

7. Bryan JP, Scheld WM. Therapy of experimental meningitis due to *Salmonella enteritidis*. *Antimicrob Agents Chemother.* 1992;36:949-954.

8. Ti T, Monteiro EH, Lam S, Lee H. Ceftriaxone therapy in bacteremic typhoid fever. *Antimicrob Agents Chemother.* 1985;28:540-543.

9. Barnaud G, Arlet G, Verdet C, Gaillot O, Lagrange PH, Philippon A. *Salmonella enteritidis*: AmpC plasmid-mediated inducible β-lactamase (DHA-1) with an *AmpR* gene from *Morganella morganii*. *Antimicrob Agents Chemother.* 1998;42:2352-2358.

10. M'Zali FH, Heritage J, Gascoyne-Ginzi DM, Denton M, Todd NJ, Hawkey PM. Transcontinental importation into the UK of *Escherichia coli* expressing a plasmid-mediated AmpC-type β-lactamase exposed during an outbreak of SHV-5 extended-spectrum β-lactamase in a Leeds hospital. *J Antimicrob Chemother.* 1997;40:823-831.

11. Pitout JD, Thomson KS, Hanson ND, Ehrhardt AF, Moland ES, Sanders CC. Beta-lactamases responsible for resistance to expanded-spectrum cephalosporins in *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis* isolates recovered in South Africa. *Antimicrob Agents Chemother.* 1998;42:1350-1354.

12. Hammami A, Arlet G, Redjeb SB, et al. Noscomial outbreak of acute gastroenteritis in a neonatal

©2000 American Medical Association. All rights reserved.

intensive care unit in Tunisia caused by multiply drug resistant *Salmonella* wien producing SHV-2 beta-lactamase. *Eur J Clin Microbiol Infect Dis.* 1991;10:641-646.
13. Gazouli M, Tzouvelekis LS, Vatopoulos AC, Tzelepi E. Transferable class C beta-lactamases in *Escherichia coli* strains isolated in Greek hospitals and characterization of two enzyme variants (LAT-3 and LAT-4) closely related to *Citrobacter freundii* AmpC beta-lactamase. *J Antimicrob Chemother.* 1998;41:119-121.
14. Bradford PA, Yang Y, Sahm D, Grope I, Gardovska D, Storch G. CTX-M-5, a novel cefotaxime-hydrolyzing beta-lactamase from an outbreak of *Salmonella typhimurium* in Latvia. *Antimicrob Agents Chemother.* 1998;42:1980-1984.
15. Tassios PT, Gazouli M, Tzelepi E, et al. Spread of *Salmonella typhimurium* clone resistant to expanded-spectrum cephalosporins in three European countries. *J Clin Microbiol.* 1999;37:3774-3777.
16. Fey PD, Safranek TJ, Rupp ME, et al. Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *N Engl J Med.* 2000;342:1242-1249.
17. Herikstad H, Hayes P, Hogan J, Floyd P, Snyder L, Angulo F. Ceftriaxone-resistant *Salmonella* in the United States. *Pediatr Infect Dis J.* 1997;16:904-905.
18. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard M7-A4.* Wayne, Pa: National Committee for Clinical Laboratory Standards; 1997.
19. Anderson ES, Ward LR, de Sax MJ, de Sa JD. Bacteriophage-typing designations of *Salmonella typhimurium*. *J Hyg (London).* 1987;78:297-300.
20. Centers for Disease Control and Prevention. *Standard Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-Field Gel Electrophoresis: CDC Training Manual.* Atlanta, Ga: Centers for Disease Control and Prevention; 1998.
21. Mathew MA, Marshall AJ, Ross GW. The use of analytical isoelectric focusing for detection and identification of β-lactamases. *J Gen Microbiol.* 1975;88:169-178.
22. Sinnert D, Richer C, Baccichet A. Isolation of stable bacterial artificial chromosome DNA using a modified alkaline lysis method. *Biotechniques.* 1998;24:752-754.
23. Sanders CC, Thomson KS, Bradford PA. Problems with detection of β-lactam resistance among non-fastidious gram-negative bacilli. *Lab Diagn Infect Dis.* 1993;7:411-423.
24. Bachman BJ. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12. In: *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology.* Washington, DC: American Society for Microbiology; 1987:1197-1219.
25. Holmberg S, Solomon S, Blake P. Health and economic impacts of antimicrobial resistance. *Rev Infect Dis.* 1987;9:1065-1078.
26. Lee C, Glenn D. Cefotaxime and ceftriaxone use evaluation in pediatrics. *Diagn Microbiol Infect Dis.* 1995;22:231-233.
27. Lee LA, Puhr ND, Maloney EK, Bean NH, Tauxe RV. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. *J Infect Dis.* 1994;170:128-134.
28. Food and Drug Administration, US Department of Agriculture, Centers for Disease Control and Prevention. *National Antimicrobial Resistance Monitoring Program: Enteric Pathogens.* Rockville, Md: Food and Drug Administration, US Dept of Agriculture, Centers for Disease Control and Prevention; 1998.
29. Winokur PL, Brueggemann A, DeSalvo DL, et al. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC β-lactamase. *Antimicrob Agents Chemother.* 2000;44:1-7.
30. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N Engl J Med.* 1998;338:1333-1338.
31. Shanon K, French G. Multiple-antibiotic-resistant *Salmonella*. *Lancet.* 1998;352:490.
32. Moosdeen F, Cheong YM. Enzymes of β-lactam resistant *Salmonella* strains. *J Antimicrob Chemother.* 1989;23:797-798.
33. Horton J, Sing R, Jenkins S. Multidrug-resistant *Salmonella* associated with AmpC hyperproduction. *Clin Infect Dis.* 1999;29:1348.
34. Bradford PA, Petersen P, Fingerman I, White D. Characterization of expanded-spectrum cephalosporin resistance in *E coli* isolates associated with bovine calf diarrheal disease. *J Antimicrob Chemother.* 1999;44:607-610.
35. US Food and Drug Administration. A proposed framework for evaluating and assuring the human safety of the microbial effects of antimicrobial new animal drugs intended for use in food-producing animals. Available at: http://www.fda.gov/cvm/index/vmac/FDAResp4_12.pdf. Accessed December 4, 2000.

Art is not a pleasure, or an amusement; art is a great matter. Art is an organ of human life transmitting man's reasonable perception into feeling.
—Leo Nikolaevich Tolstoy (1828-1910)

©2000 American Medical Association. All rights reserved.